

WHAT IS CLAIMED IS:

1. A method of analyzing prokaryotic gene expression comprising the processes of:

an mRNA isolation process for isolating an mRNA from a
5 prokaryotic cell;

a polyA addition process for adding a polyA at the 3' end of the mRNA;

a cDNA synthesis process for synthesizing a cDNA from the polyA-added mRNA;

10 a cDNA processing process for preparing an adaptor-attached cDNA fragment having the sequence of a first adapter at one end and the sequence of a second adapter at the other end, from the cDNA;

a first PCR process for performing PCR with the
15 adaptor-attached cDNA fragments, using a first primer having a sequence complementary to the sequence of the first adaptor and a second primer having a sequence complementary to the sequence of the second adaptor;

an electrophoresis process for performing
20 electrophoresis with the cDNA fragments amplified in the first PCR process; and

a cDNA fragment recovery process for recovering the desired cDNA fragment based on the result of the electrophoresis.

2. A method of analyzing prokaryotic gene expression according to Claim 1, wherein the mRNA isolation process comprises:

5 a process of isolating the whole RNA from the prokaryotic cell;

a process of hybridizing a first nucleotide having a sequence complementary to a portion of 16S rRNA with the 16S rRNA, and simultaneously hybridizing a second nucleotide having a sequence complementary to a portion of 23S rRNA with
10 the 23S rRNA;

a process of hybridizing a first tag substance Tag Substance 1) to which is added a third nucleotide having a sequence complementary to a site that is different from the site complementary to the 16S rRNA in the first nucleotide,
15 with the hybrid of the 16S rRNA and the the first nucleotide, and simultaneously hybridizing a second tag substance to which is added a fourth nucleotide having a sequence complementary to a site that is different from the site complementary to the 23S rRNA in the second nucleotide, with
20 the hybrid of the 23S rRNA and the second nucleotide; and

a process of removing the hybrid of the 16S rRNA, the first nucleotide and the first tag substance added with the third nucleotide, and simultaneously removing the hybrid of the 23S rRNA, the second nucleotide and the second tag
25 substance added with the fourth nucleotide, from the whole

RNA.

3. A method of analyzing prokaryotic gene expression according to Claim 2, wherein

5 the first nucleotide and the second nucleotide are identical ones having a sequence complementary to the common sequence present in both 16S rRNA and 23S rRNA,

the third nucleotide and the fourth nucleotide are also identical, and

10 the first tag substance and the second tag substance are also identical.

4. A method of analyzing prokaryotic gene expression according to Claim 1, wherein

15 the cDNA synthesis process comprises the synthesis of the cDNA as well as the addition of a tag substance at the 5' end of the cDNA at the same time; and

the cDNA processing process comprises:

20 a first cleavage process for cleaving the cDNA by means of a type I restriction enzyme;

a first recovery process for recovering the cDNA fragments having the tag substance by binding a high-affinity substance having high affinity to the tag substance;

25 a binding process of the first adaptor for binding to the cDNA fragments having the tag substance, the sequence of

the first adaptor having a sequence complementary to the sequence at the cleavage site of the type I restriction enzyme;

5 a second cleavage process for cleaving the cDNA fragment bonded with the sequence of the first adaptor by means of a type II restriction enzyme;

a second recovery process for removing the cDNA fragments with the tag substance and recovering the cDNA fragment not having the tag substance, by binding them with
10 the high-affinity substance; and

a binding process of the second adaptor for binding to the cDNA fragment not having the tag substance, the sequence of a second adaptor having a sequence complementary to the sequence at the cleavage site of the type II restriction
15 enzyme.

5. A method of analyzing prokaryotic gene expression according to Claim 1, wherein

the electrophoresis process is carried out by means of
20 gel electrophoresis; and

the cDNA fragment recovery process is carried out by cutting out the portion of gel containing the desired cDNA fragment from the gel and recovering the corresponding cDNA fragment.

6. A method of analyzing prokaryotic gene expression according to Claim 1, wherein at least one part of the first primer and the second primer is(are) given with a marker substance, and the marker substance is detected in the
5 electrophoresis.

7. A method of analyzing prokaryotic gene expression according to Claim 4, wherein the combination of the tag substance and the high-affinity substance is any one of the
10 combinations of biotin and streptavidin, of biotin and avidin, of FITC and FITC antibody, and of DIG and anti-DIG.

8. A method of analyzing prokaryotic gene expression according to Claim 1, which comprises, after the cDNA
15 fragment recovery process,

a ligation process for ligating the recovered cDNA fragment to a plasmid vector to form a recombinant plasmid;
and

an incorporation process for incorporating the
20 recombinant plasmid into Escherichia coli.

9. A method of analyzing prokaryotic gene expression according to Claim 8, which comprises, after the cDNA fragment recovery process and before the ligation process, a
25 second PCR process for performing PCR with the recovered cDNA

fragment, using a third primer having a sequence complementary to the sequence of the first adaptor and a fourth primer having a sequence complementary to the sequence of the second adaptor.